Lights and shadows: growth patterns in three sympatric and congeneric sponges (*Ircinia* spp) with contrasting abundances of photosymbionts

Xavier Turon[1,a], Anna Garriga[2], Patrick M. Erwin[2b]

[1]Centre for Advanced Studies of Blanes (CEAB-CSIC). Accés Cala S Francesc 14, 17300 Blanes (Girona), Spain

[2]Dept. of Animal Biology. University of Barcelona. 647 Diagonal Ave., 08028 Barcelona, Spain

[a]Corresponding author:

E-mail: xturon@ceab.csic.es

[b]Current address:

Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Ln., Wilmington, NC 28409, USA

Abstract

The life-history traits of long-lived benthic littoral invertebrates remain poorly understood. In this study, we analysed patterns of growth in three abundant sublittoral sponges from the western Mediterranean Sea, chosen for their close phylogenetic relatedness, sympatric distribution, and contrasting amounts of photosymbionts: high in *Ircinia fasciculata*, lower in *I. variabilis*, and absent in *I. oros*. Sponge area, perimeter, number of oscula and epibiont abundance were quantified from *in situ* digital images taken monthly for 1.5 years and volumetric growth rates were calculated from empirical area-volume relationships. Volumetric growth rates were different among species and coherent with the photosymbiont abundance: high in *I. fasciculata* (40.03 ±4.81% yr^{-1}, mean ±SE), low in *I. variabilis* (5.65 ±6.11% yr^{-1}) and almost nil in *I. oros* (-0.04 ±3.02% yr^{-1}). Furthermore, a marked seasonality was observed in the first two species, with greater growth during the warm season. The high growth rates of *I. fasciculata* were likely fuelled by symbiont-derived photosynthates and required to compete in the well-lit, algal-dominated habitats this species prefers. In contrast, *I. variabilis* and *I. oros* tended to dwell in shaded habitats, where competition from slow-growing invertebrates is intense, and featured lower growth rates. The flattened morphology and lower circularity of *I. variabilis* indicates a capacity for adaptation to any space that is freed, while *I. oros* had less oscula and was more massive and circular, suggesting a strategy of passive occupation and minimization of biological interactions. The results show that even congeneric species living sympatrically can achieve important biomass using different growth and substrate occupation strategies.

Introduction

Long-lived benthic invertebrates generally exhibit slow growth dynamics and represent a stable component and an important fraction of the biomass and structural complexity of littoral systems (McMurray et al. 2008; Teixidó et al. 2011). These long-lived and structure-forming species are at the same time vulnerable elements of these ecosystems, with human alteration as well as diseases heavily impacting species with inherently low capacities for fast recovery (Coma et al. 2009; Teixidó et al. 2011). There is a general scarcity of basic knowledge of these organisms, in part due to the need for long monitoring periods and to inter-individual variability in many parameters (Reiswig 1973; Turon and Becerro 1992; Garrabou and Zabala 2001). Life cycle parameters such as growth and regeneration rates, shape and size changes, and mortality, among others, are determining factors for the function and interactions among species, yet surprisingly few species have been studied from a basic life-history perspective.

Sponges in particular are slow-growing organisms that constitute a major component of the invertebrate fauna in the rocky sublittoral. In terms of diversity and biomass, they are second only to corals in coral reef systems (even comparing favourably with them, Diaz and Rützler 2001; Rützler 2012) and are among the dominant groups in temperate and high-latitude hard-bottom communities (e.g., McClintock et al. 2005; Van Soest et al. 2012; Wulff 2012). Yet basic life-history data is wanting for most species and growth rates have only been investigated for a few species, mostly of encrusting morphology (e.g., Ayling 1983; Pansini and Pronzato 1990; Turon et al. 1998; Garrabou and Zabala 2001; Tanaka 2002; De Caralt et al. 2008). Fewer studies still have addressed the growth of more three-dimensional (massive) sponges, partly due to technical hurdles for accurate assessment of volume (e.g., Wulff 1985; Hoppe 1988; Trusell et al. 2006; Koopmans and Wijfels 2008; McMurray et al. 2008; Rohde and Schupp 2012). In addition to studies specifically devoted to growth rate measurements, a literature perusal shows that growth data is also provided in studies addressing other aspects of sponge biology, such as mutualism or predation resistance (e.g., Ellison et al. 1996; Wulff 1997, 2005,

2008). Even so, the number of species for which growth information is available is clearly too low considering the diversity and ecological importance of sponges.

Sponges are also known to harbour a highly diverse array of prokaryote symbionts (reviewed in Webster and Taylor 2011; Thacker and Freeman 2012), among which are both photosynthetic and heterotrophic microbes. As the diversity of such symbionts begins to be well characterised, knowledge of the implications of these associations for the sponges' biology and interactions is increasing (Taylor 2007; Webster and Taylor 2011, Fan et al. 2012, Liu et al. 2012). In particular, the presence of photosymbionts is widespread and can potentially influence growth rates through phototrophic nutrition, yet little experimental evidence exists documenting the potential contribution of photosymbionts to sponge growth (e.g., Arillo et al. 1993; Thacker 2005; Erwin and Thacker 2008; Freeman and Thacker 2011).

Keratose sponges of the genus *Ircinia* are important components of benthic communities in the sublittoral of the Mediterranean Sea (Uriz et al. 1992; Martí et al. 2004; Cebrian et al. 2011). Notwithstanding this ecological relevance, there is an almost complete lack of basic biological information, possibly because they are not commercially valuable (Maldonado et al. 2010). Species of the genus *Ircinia* (and related genera) have been notorious in recent times because they have suffered important mortality episodes in the Mediterranean (Vacelet et al. 1994; Perez et al. 2000; Stabili et al. 2012), as well as recurrent partial mortality (Maldonado et al. 2010), possibly related to episodes of anomalously high seawater temperatures (Coma et al. 2009; Maldonado et al. 2010). Interestingly, the susceptibility of *Ircinia* species harbouring cyanobacteria may be higher than that of related, non-photosymbiotic species (Cebrian et al. 2011). Important losses of *Ircinia* spp have also occurred in other areas, such as the Caribbean (Wulff 2006, Stevely et al. 2011).

We have chosen to study a system comprised of three species of *Ircinia*, namely *I. fasciculata* (Pallas 1766), *I. variabilis* (Schmidt 1862), and *I. oros* Schmidt 1864), that are closely related phylogenetically (Erwin et al. 2012a),

have a similar massive growth form, and coexist in the same localities, albeit with different ecological preferences. Thus, many evolutionary (host-related) factors and environmental conditions are shared among these species, rendering them an ideal model to disentangle species-specific characteristics from environmental factors. Further, the structure and dynamics of their symbiont communities have been recently studied in detail (Erwin et al. 2012 a,b,c) and feature contrasting amounts of photosymbionts: high in *I. fasciculata,* lower in *I. variabilis* (the amount of chlorophyll *a* in *I. variabilis* is ca. one half that of *I. fasciculata*, Erwin et al 2012b,c), and absent in *I. oros*. As we lack baseline biological information on these important and threatened members of sublittoral Mediterranean communities, the aim of the present work was to analyse growth patterns and other biological parameters of three sympatric and congeneric *Ircinia* species, complementing previous studies of the symbiont dynamics (Erwin et al. 2012c) and relating the patterns found to environmental variables and intrinsic features of the species studied.

Materials and methods

Study sites and sampling

We analysed sponges from two nearby (ca. 12 km apart) localities in the Western Mediterranean: “Punta de S’Agulla” (Blanes; 41°40′54.87″ N, 2°49′00.01″ E) and “Mar Menuda” (Tossa de Mar; 41°43′13.62″ N, 2°56′ 26.90″ E). Individual sponges were mapped *in situ* and, where necessary, marked with epoxy tags placed near the sponges. Individuals of *I. fasciculata* (*n* = 25) were monitored in S’Agulla and individuals of *I. variabilis* (*n* = 24) and *I. oros* (*n* = 26) were studied in Tossa de Mar (Fig. 1). The communities in both localities included all three species, but monitoring sites for each species were chosen based on their respective abundances in each locality. Both localities featured similar habitats with shallow sublittoral rocky communities on vertical and subvertical walls. Although the three sponge species can occur side by side, there was some degree of ecological zonation among these species within each locality. *I. fasciculata* preferentially occurs in more exposed and well-lit habitats,

where seasonal erect algae dominate, while *I. variabilis* and *I. oros* dwell in more shaded habitats, usually on vertical walls, where crustose algae and other invertebrates are dominant. More detailed descriptions of the benthic communities found in Tossa de Mar is given in Turon (1990) and Palacin et al. (1998). Due to these different ecological preferences, *I. fasciculata* was sampled at shallower (3 to 7.2 m) depths than the other two species (7 to 16.1 m). Sponges were selected haphazardly without regard to their size, in order to obtain a representative range of all size classes for the three species in these populations (initial sizes were 65.441 to 87.657 cm^3 for *I. fasciculata*, 9.401 to 14.764 cm^3 for *I. variabilis*, and 69.104 to 87.433 cm^3 for *I. oros*). HOBO® (Onset Computer Corporation) dataloggers were deployed at each locality to register hourly data on seawater temperature.

Sponges were photographed monthly from March 2010 to August 2011. For *I. oros*, the monitoring period started in May 2010 as this species was initially monitored in another nearby locality that had to be abandoned due to the onset of heavy construction in an adjacent artificial breakwater. A digital camera with underwater housing coupled to a fixed frame was used to ensure the same focal distance in all pictures. The pictures were taken perpendicularly to the substratum to measure total area covered by each sponge specimen. Additionally, samples from each species (n = 36 to 38) encompassing the size ranges of the studied sponges were collected from the same or adjacent localities to determine empirical area-volume relationships. These samples were transported to the laboratory and photographed with the same camera, housing and frame to obtain their area. The volume of each sponge was then measured by suspension in distilled water placed on a scale, following the removal of foreign macroorganisms. The resulting change in weight registered by the scale corresponded to the weight of water displaced by the sponge, from which the sponge volume was obtained.

Digital image processing and measurements

Calibrated digital photographs were processed in the image analysis software ImageJ v1.46 (NIH) using a graphic slate to trace the outline of each sponge

and measure sponge area and perimeter. The number of oscula visible in each image was also counted. To quantify the abundance of macroscopic fouling organisms (epibionts), a digitized 10 x 10 grid was superimposed over each image and adjusted to fit the sponge specimen as much as possible. Then, 10 squares of the grid were randomly chosen using a random number generator and scored for the presence (1) or absence (0) of epibionts.

Calculations and data analysis

Sponge area-volume relationships

The area-volume relationship for each sponge species was obtained from empirical data fitted to the power regression: volume = a * area^b, where a and b are parameters obtained using the nonlinear module of the Systat v. 12 program. Power regression produced better fit than linear or exponential models and consistently high correlation coefficients (>0.98, see Results), allowing for reliable transformation of sponge areas into sponge volumes.

Growth rates

Growth rates (GR) were calculated on a monthly basis from the equation:

$$GR_m = \frac{V_m - V_{(m-1)}}{V_{(m-1)}}$$

Where V_m is the volume at month m and $V_{(m-1)}$ the volume in the previous month. To reduce noisy fluctuations, when drawing the plots growth rates were smoothed using a weighed moving window (Turon et al. 1998), as follows:

$$GR_{smoothed} = GR_m \times 0.5 + GR_{(m-1)} \times 0.25 + GR_{(m+1)} \times 0.25$$

Oscula density

Number of oscula per volume was calculated for each sponge individual during each monitoring month. As there were no consistent differences over time in

this parameter, monthly values were averaged to obtain a single value per sponge individual and allow for intra- and interspecies comparisons.

Circularity index

The circularity index (CI, Becerro et al. 1994) was calculated on a monthly basis from the equation:

$$CI = \frac{A}{\pi * \left(\frac{P}{2\pi}\right)^2}$$

Where A and P are the sponge area and perimeter, respectively. This index measures the relationship between the observed area and the area of a circle with the equivalent perimeter, resulting in a value of 1 for a perfect circle and decreasing as the outline becomes more irregular. As there were no consistent differences over time, monthly values were averaged to obtain a single value per individual for intra- and interspecies comparisons.

Epibiosis Index

The epibiosis index was calculated on a monthly basis for each sponge individual as the percentage of grid squares (see above) containing macroscopic epibionts.

Statistical tests

Area-volume relationships were compared among sponge species with Analysis of Covariance (ANCOVA), first testing the homogeneity of slopes in a model with an interaction term, and then assessing intercept differences between species in a reduced model (no interaction term).

To assess differences in growth rates, oscula density, circularity and epibiosis among sponge species, parametric analysis of variance (ANOVA) or,

when the assumptions of normality and homogeneity of variance were not met, Kruskal-Wallis (K-W) tests were conducted. A posteriori pairwise comparisons were made with Student-Newman-Keuls test (ANOVA) and Dunn test (K-W).

Pearson correlation tests were performed to relate mean individual growth rates with: initial area, oscula density, circularity, and epibiosis. Likewise, cross-correlation analyses were performed to assess relationships between mean monthly growth rates and temperature for each species. In cross-correlation analysis, two time series are compared using the Pearson correlation coefficient with an increasing lag of one series with respect to the other. Correlation at time lag 0 is the usual Pearson correlation. Correlations at positive lags analyze relationships of the values in the first series with values of the second series that number of lags afterwards. Correlations at negative lags relate values in the first series with previous values in the second series.

Statistical analyses were performed with the software program Sigmastat v.3.2, except for ANCOVA (Statistica v 6.0) and cross-correlations (Systat v. 12).

Results

Area-volume relationships

The fitting of a power function to area-volume relationships yielded high correlation coefficients in the three species ($r > 0.98$, Fig. 2), allowing for high confidence estimation of sponge volume based on area calculations. The growth was negatively allometric (exponent < 3/2) for all species, indicating that the shape varied with growth, with an increase in area higher than the corresponding increase in volume under isometric growth. Thus, sponges tended to adopt a more flattened shape as they grew.

Statistical analysis of area-volume relationships (ANCOVA) with a complete model showed a highly significant species*area interaction (Table 1), indicating that the shape of the relationship between area and volume varied according to the species. Pairwise comparisons revealed that the difference

was attributable to *I. variabilis*, which exhibited a significantly different slope of the area-volume relationship ($P < 0.001$), as indicated by a lower exponent ($b = 1.211$). In other words, *I. variabilis* tends to adopt a more flattened morphology with increasing area than the other two species (Fig 2). On the other hand, the comparison of *I. fasciculata* and *I. oros* did not show a significant interaction ($P = 0.113$) and a reduced model without an interaction term for these two species showed also no differences in intercepts of the area-volume relationships (Table 1, factor species, $P = 0.611$). Thus, the pattern of volumetric growth for *I. fasciculata* and *I. oros* was similar (Fig. 2d).

Growth rates, seasonality and mortality

Overall, the monitored specimens of *I. fasciculata* grew actively during the study period, with a mean annual volumetric growth rate of 0.400 ±0.048 (mean ±SE), while *I. variabilis* exhibited a lower annual growth rate (0.056 ±0.061) and *I. oros* featured an overall negative (though close to 0) growth rate (-0.0004 ±0.030). Annual growth rates were significantly different among species (ANOVA, $P < 0.001$) and multiple pairwise comparisons (SNK test) revealed that *I. fasciculata* had a significantly higher growth than the other two species, which did not show significant differences among them.

Individuals of *I. fasciculata* also exhibited a seasonal pattern of higher growth in late Spring and Summer (Fig. 3). The growth rates over the study period showed positive growth (i.e., GR > 0) at all times with the exception of the coldest months at the end of winter. Interannual differences were also observed, as growth rates were higher during the first warm season. Further, cross-correlation analyses of mean monthly growth rates with temperature revealed a close relationship between both parameters for *I. fasciculata* (Fig. 4). There were significant positive correlations at time lag 0 (i.e., contemporary values of the two series), a relationship that decreases as one series is lagged with respect to the other, reaching minimal values at 5-6 months of lag, in a pattern typical of parameters that oscillate seasonally.

Individuals of *I. variabilis* displayed a similar seasonal pattern of growth to *I. fasciculata* (Fig. 3), with higher GR during the warm periods and arrested or negative growth extending from late Autumn to mid-Spring. Cross-correlation analyses also revealed a similar relationship between monthly GR and temperature for *I. variabilis* (Fig. 4), with correlations that turn from positive to negative as growth rate is compared with temperature in the previous or subsequent months.

Individuals of *I. oros* exhibited a markedly different course and pattern of growth (Fig. 3), with high variability even between consecutive months and no clear seasonal patterns. Minimal GR values were observed at the end of Winter (2011) and GR were mostly positive during the first half of the study, and mostly negative during the second half. In cross-correlation analyses (Fig. 4), no significant correlation between GR and temperature was found at any time lag, and the highest values were found between growth rates and temperatures 3-4 months before (negative lags).

During the study period, no significant mortality was observed, with only one instance of partial mortality in *I. fasciculata*, one partial and two total mortality events in *I. variabilis*, and no mortality events in *I. oros*. By partial mortality, we refer to a significant loss (>50%) of biomass, leaving the dead skeleton, but with some part of the colony surviving. In the case of total mortality, the skeleton could be seen at the next observation time, before it finally detached from the substrate.

Relationships of growth rates with other parameters

The relationship of the growth rates with initial size of the sponges (Fig. 5) revealed that only *I. fasciculata* had a pattern of decreased growth for larger sponge individual, although this relationship was not significant (r = -0.383, P = 0.086). On the contrary, *I. variabilis* and *I. oros* exhibited a positive, albeit not significant, relationship (r = 0.182, P = 0.551 and r = 0.100, P = 0.684, respectively). The same analyses performed with mean, instead of initial, size of the sponges yielded consistent results (not shown).

The average number of oscula per unit volume showed important differences among the three species (Fig. 6), with *I. variabilis* featuring the highest values (0.661 ±0.088 oscula cm^{-3}, mean ±SE), followed by *I. fasciculata* (0.187 ±0.019 oscula cm^{-3}) and *I. oros* (0.081 ±0.007 oscula cm^{-3}). The differences between the three species were significant for all pairwise comparisons (K-W test, H = 31.211, *df* = 2, $P < 0.001$; Dunn test, all comparisons $P < 0.01$). The number of oscula per volume was negatively correlated with the mean sponge volume in *I. fasciculata*, with marginal significance ($r = -0.414$, $P = 0.062$), and uncorrelated with volume in the other two species (*I. variabilis*, $r = -0.467$, $P = 0.108$; *I. oros*, $r = 0.161$, $P = 0.510$). On the other hand, there was a significant negative relationship between growth rate and number of oscula for *I. oros* ($r = -0.486$, $P = 0.035$), while there was no significant correlation in the other two species (*I. fasciculata*, $r = -0.135$, $P = 0.561$; *I. variabilis*, $r = 0.134$, $P = 0.662$).

Significant differences among sponges were also observed for the circularity index (Fig. 7, K-W test, H = 28.247, df = 2, $P < 0.001$), with *I.* oros exhibiting significantly higher values than the other two species (Dunn test, $P < 0.01$). This indicates that *I. oros* tended to have a more circular outline overall. There was no relationship between circularity of the specimens and growth rate for any species (*I. fasciculata*, $r = -0.151$, $P = 0.514$; *I. variabilis*, $r = 0.222$, $P = 0.466$; *I. oros*, $r = 0.111$, $P = 0.650$).

Overall, the epibiosis index values were high for all three sponge species (above 50% in most cases, Fig. 8). *I. oros* exhibiting the highest mean values over the study period (75.8 ±2.9%, mean ±SE), followed by *I. variabilis* (67.5 ±4.9%) and *I. fasciculata* (63.7 ±3.9%), although differences among species were not significant (K-W test, H = 4.244, df = 2, $P = 0.120$). The analysis of fouling over time showed that, in *I. fasciculata*, the lowest degree of epibiosis was found during the warmer months and fouling tended to increase with decreasing temperatures (Fig. 8). In *I. variabilis*, the values were more homogeneous through time, but with minima during the first summer (Fig. 8). For *I. oros* the pattern differed between years, with higher values of fouling during the second half of the study, although a decrease with increasing temperatures could also be observed at both years (Fig. 8). No significant

correlation between GR and epibiosis occurred for any species (*I. fasciculata*, r = 0.169, P = 0.464; *I. variabilis*, r = 0.275, P = 0.364; *I. oros*, r = -0.138, P = 0.574).

Discussion

The studied sponges represented phylogenetically closely-related species (genus *Ircinia*) and occurred sympatrically, yet showed markedly different growth strategies. *I. fasciculata* and *I. variabilis* grew in general during the warm season (from June to October) and showed reduced growth, or even regression, during the coldest months (November-May). For *I. oros*, however, no clear seasonality was found. Overall growth rates were also different among species, an order of magnitude higher in *I. fasciculata* (ca. 40% in volume per year) than in *I. variabilis* (ca. 5%), and ca. zero in *I. oros*. We highlight here that well-developed sponges were monitored in our study to get a representation of the size-classes most common in the area. Our results, therefore, may not reflect the initial growth of recently settled and juvenile sponges, but rather the situation once they have reached a size at which available space has been occupied and interactions with neighbours are well established. Accordingly, this may explain why no relationship between initial volume of sponges with growth rate was observed, while previous studies have shown that, in sponges, smaller specimens usually grow faster (e.g., Hoppe 1988; De Caralt et al. 2008; Teixidó et al. 2009; Rohde and Schupp 2012).

Growth patterns related well with the abundance of photosymbionts in each species, high in *I. fasciculata*, lower in *I. variabilis*, and absent in *I. oros* (Erwin et al. 2012a, 2012c). The microbial communities (including cyanobacteria) that inhabit these sponges are remarkably stable over different seasons in terms of their structure, although seasonal fluctuations in chlorophyll *a* content may indicate variable photosynthetic output from the stable symbiont communities (Erwin et al. 2012c). Phototrophic nutrition may contribute to the growth of *I. fasciculata* and *I. variabilis* (Erwin et al. 2012b), as observed in other sponge-cyanobacteria symbioses (Thacker 2005; Erwin and Thacker

2008; Freeman and Thacker 2011). This suggestion will require further confirmation, as we do not have direct measures of photosynthetic efficiency of the monitored specimens. While the growth rates of these two species were significantly correlated with temperature, we cannot discard that irradiance (and by extension, photosymbionts) was also an underlying relevant factor for the seasonality observed since irradiance also varies seasonally in the studied habitats, both in day-length and intensity (higher in Spring-Summer) (Mariani et al 2005; Erwin et al. 2012c). Experimental work should be undertaken to disentangle the role of light and temperature in determining growth rates and seasonality in *Ircinia* species with photosymbionts. We should also consider the potential role of the abundant heterotrophic bacteria in the studied species. Among other functions, they could provide chemical defences and fix nitrogen for the hosts, thus having a relevant role in growth rates. Assumptions of mutualism need to be corroborated by manipulative experiments (e.g., growth under different light conditions) and compound translocation analyses. In a study coupling both approaches, Freeman & Thacker (2011), found a complex pattern of species-specific interactions. Our system will surely prove a good model for experimentally assessing benefits and costs of sponge-microbe associations.

In previous studies, Mediterranean sponges have shown low growth rates in general (e.g., Pansini and Pronzato 1990; Turon et al. 1998; De Caralt et al. 2008; Teixidó et al. 2009), along with long life-spans and low mortality rates. In fact, some sponges did not show significant growth in as much as 25 years of observations (Teixidó et al. 2011), while the indeterminate nature of sponge growth was reflected by the succession of growth and regression in other species (e.g., Turon et al. 1998, Garrabou and Zabala 2001). Even if our study covers a restricted temporal frame (18 mo), our results agree with this general view of slow dynamics, low mortality, and occasional regression in Mediterranean sponges. If any, *Ircinia* species featured slower growth rates than those reported for encrusting sponges in the NW Mediterranean (115% yr^{-1}, Turon et al. 1998; 26-240% yr^{-1}, Garrabou and Zabala 2001; 228% yr^{-1}, De Caralt et al. 2008), which can be attributed to the higher scope for growth in thinly encrusting species whenever free space becomes available. Sponge

412 dynamics can be further complicated by colony fission and fusion events,
413 especially in encrusting forms (e.g., Turon et al. 1998; Teixidó et al. 2009) or by
414 fragment dynamics in branching species (Wulff 1991), but in our massive
415 growth-form species no instance of fusion or fragmentation was found.

416 Little information is available on growth of species of *Ircinia*. The
417 Caribbean species, *I. strobilina*, which lacks photosymbionts, had volumetric
418 growth rates of ca. 10% on an annual basis, within the range reported herein.
419 On the other hand, attempts at sponge culture for biotechnological applications
420 using fragments of *I. variabilis* showed high growth rates (up to 200% yr^{-1}, Van
421 Treeck et al. 2003). However, the growth of fragments under culture conditions
422 cannot be considered a realistic picture of what happens in the natural habitat of
423 the sponges (Koopmans and Wijffels 2008) and may exaggerate growth rates
424 due to excessive wound healing processes (tissue regeneration), which
425 generally exceed natural growth processes (Ayling et al. 1983; Hoppe 1988).

426 It has been reported that summer is the limiting season for many filter-
427 feeding invertebrates in the Mediterranean, due to water stratification and
428 resource limitation (Coma et al. 2000, 2009). In our case, the opposite was
429 found. The species that featured clear seasonal growth (*I. fasciculata* and *I.*
430 *variabilis*) grew actively during the warmest months, as described for other
431 Mediterranean sponges (Turon et al. 1998; De Caralt et al. 2008; Di Camillo et
432 al. 2012). Higher irradiance, longer day-length and lower fouling levels during
433 the warm season may contribute, via enhanced activity of photosymbionts, to
434 the lack of resource limitation during summer in the species studied herein.

435 Alternatively, the seasonality observed in growth patterns may be the
436 result of a temporal trade-off with resource allocation to other functions (Sebens
437 1987). In particular, growth and reproduction have been shown to be temporally
438 segregated in some invertebrates (e.g., López-Legentil et al. 2005; Pérez-
439 Portela et al. 2007). Although we lack detailed studies on the reproduction of
440 the three *Ircinia* species considered, they all brood larvae during summer
441 (Mariani et al 2005, and pers. obs.) and energy devoted to gamete production
442 and larval rearing would be highest in the preceding months (McMurray et al
443 2008, Pérez-Porro et al 2012). For *I. fasciculata* and *I. variabilis*, this

444 reproductive investment roughly coincides with the period of growth reactivation
445 after winter and thus provides no clearcut evidence for resource partitioning
446 between growth and reproduction in these species. The absence of a trade-off
447 between growth and reproduction has also been found in other sponge species
448 (Ayling et al 1983, McMurray et al 2008, Di Camillo et al 2012).

449 We investigated the potential relationship between number of oscula per
450 volume unit and growth rates. Other things being equal, it is conceivable that a
451 higher number of oscula correlates with a greater capacity for water pumping,
452 particle filtration and thus growth. However, the results did not support this idea
453 as *I. variabilis* had by far the highest number of oscula but not the highest
454 growth rate. For all three species, growth rates and number of oscula were not
455 correlated (*I. fasciculata* and *I. variabilis*) or were negatively correlated (*I. oros*).
456 Thus, the parameter chosen may be too simplistic and other features of the
457 architecture of the aquiferous system that vary between and within species may
458 be more informative for comparisons with growth rates.

459 The growth form and morphology of benthic invertebrates have important
460 implications in space competition, as the perimeter marks the zone of
461 competitive interaction with neighbours (Turon et al. 1996). In some cases,
462 sponge shape has been shown to be plastic and vary as a function of the nature
463 of neighbours (Becerro et al. 1994). In this sense, more circular outlines, as
464 observed for *I. oros* herein, may reflect a strategy of minimization of interactions
465 at the expense of not being able of directional growth allowing occupation of
466 free space (Becerro et al. 1994). In contrast, the more flattened growth form of *I.*
467 *variabilis* suggests an alternative strategy: greater flexibility in the occupation of
468 newly freed substrate at the expense of greater competitive interactions with
469 neighbours.

470 The epibiosis observed in the sponges followed roughly seasonal trends,
471 masked by marked interindividual differences in *I. fasciculata* and *I. oros*, while
472 more constant in *I. variabilis*. Some sponges were permanently fouled
473 throughout the observation period, while others were only rarely seen with
474 macroepibionts. Sponge epibionts belonged to different groups (mostly algae,
475 but also bryozoans, hydroids and serpulids), some with observable seasonal

trends in abundance, such as an increase in red algae during the winter and green algae during summer. Overall, the lowest values in the three species were found in summer, which was unexpected given algal proliferation at this season. The levels of epibiosis were surprisingly high given that these species are chemically-rich. Although taxonomic controversies (Erwin et al 2012a) make it difficult to ascertain which compounds are particular to each species, there are a number of compounds described for them (e.g. Cimino et al. 1972, Alfano et al. 1979), and their metabolites can have antifouling activity (Amade et al. 1987; Tsoukatou et al. 2002). It should be noted that epibiosis rates were likely overestimated by our method, as whenever a macroepibiont was recorded in a selected square, this square was assigned a value of 1, even if the epibiont itself did cover the square only partially. No relationship between fouling degree and growth rate were observed among the species investigated, suggesting that the presence of epibionts did not represent a major nuisance for sponge growth.

In conclusion, the growth dynamics of the three species studied suggest ecological different strategies for substrate occupation and survival. *I. fasciculata* preferentially inhabits well-lit and shallow habitats, features a high density of cyanobacteria (Erwin et al. 2012a, c) and exhibited the highest growth rates in spite of having the lowest number of oscula per sponge volume. This indicates that photosynthates derived from symbionts may fuel the growth of *I. fasciculata* and allow this species to successfully compete in algal-dominated habitats. *I. variabilis* and *I. oros* occurred in the study area in more shaded places and at greater depths and featured low numbers (*I. variabilis*) or the absence altogether (*I. oros*) of photosymbionts. Their growth rates were much slower than that of *I. fasciculata*, suggesting different strategies to compete in habitats dominated by slow-growing invertebrates (Turon et al. 1996). With the highest number of oscula, a more flattened shape and a less circular outline, *I. variabilis* appears to utilize its high filtration capacity and adapt to spaces generated along the periphery of the sponges. With the slowest (in fact, slightly negative overall) growth rate, lowest abundance of oscula and more circular outlines, *I. oros* appears to adopt a strategy of slow growth and passive occupation of substrate by minimizing interactions.

In spite of these differences, all three species are long-lived, slow-growing and featured low mortality during the study period. As abundant, structure-forming and stable members of the sublittoral communities, these sponges are both ecologically relevant and vulnerable to environmental changes (Coma et al. 2009; Teixidó et al. 2011), as exemplified by recent outbreaks of diseases and mortality episodes in *Ircinia* spp in the Mediterranean and elsewhere (Wulff 2006; Maldonado et al. 2010; Cebrian et al. 2011; Stevely et al. 2011). The proximate cause of these outbreaks may be bacterial proliferation (Stabili et al. 2012), but the ultimate cause is likely attributable to global warming and prolonged phases of water stratification (Coma et al. 2009). Fortunately, the sponges in the zone studied are not (yet) impacted by these diseases and provide baseline biological information that may prove invaluable for the future assessment of their health and the status of Mediterranean sublittoral ecosystems in the face of a changing climate.

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References

Alfano G, Cimino G, De Stefano S (1979) Palinurin, a new linear sesterpene from a marine sponge. Experientia 35:1136-1137

Amade P, Charroin C, Baby C, Vacelet J (1987) Antimicrobial activities of marine sponges from the Mediterranean Sea. Mar Biol 94:271-275

536 Arillo A, Bavestrello G, Burlando B, Sarà M (1993) Metabolic integration between
537 symbiotic cyanobacteria and sponges: a possible mechanism. Mar Biol 117: 59-
538 162

539 Ayling AM (1983) Growth and regeneration rates in thinly encrusting demospongiae
540 from temperate waters. Biol Bull 165:343-352

541 Becerro MA, Uriz MJ, Turon X (1994) Trends in space occupation by the encrusting
542 sponge *Crambe crambe*: variation in shape as a function of size and
543 environment. Mar Biol 121:301-307

544 Cebrian E, Uriz MJ, Garrabou J, Ballesteros E (2011) Sponge mass mortalities in a
545 warming mediterranean sea: are cyanobacteria-harboring species worse off?
546 PLoS One 6(6):e20211

547 Cimino G, De Stefano S, Fattoruso E, Minale L (972) Ircinin-1 and -2 linear
548 sesterterpenes from the marine sponge *Ircinia oros*. Tetrahedron 28:333-341

549 Coma R, Ribes M, Gili JM, Zabala M (2000) Seasonality in coastal benthic
550 ecosystems. Trends Ecol Evol 15:448-453

551 Coma R, Ribes M, Serrano E, Jiménez E, Salat J, Pascual J (2009) Global warming-
552 enhanced stratification and mass mortality events in the Mediterranean. P Natl
553 Acad Sci USA 106:6176-6181

554 De Caralt S, Uriz MJ, Wijffels RH (2008) Grazing, differential size-class dynamics and
555 survival of the Mediterranean sponge *Corticium candelabrum*. Mar Ecol Prog
556 Ser 360:97-106

557 Diaz MC, Rützler K (2001) Sponges: an essential component of Caribbean Coral
558 Reefs. Bull Mar Sci 69:535-546

559 Di Camillo CG, Coppari M, Bartolucci I, Bo M, Betti F, Bertolino M, Calcinai B, Cerrano
560 C, De Grandis G, Bavestrello G (2012) Temporal variations in growth and
561 reproduction of *Tedania anhelans* and *Chondrosia reniformis* in the North
562 Adriatic Sea. Hydrobiologia 687:299-313

563 Ellison AM, Farnsworth EJ, Twilley RR (1996) Facultative mutualism between red
564 mangroves and root-fouling sponges in Belizean mangal. Ecology 77: 2431-
565 2444

566 Erwin PM, Thacker RW (2008) Phototrophic nutrition and symbiont diversity of two
567 Caribbean sponge-cyanobacteria symbioses. Mar Ecol Prog Ser 362:139-147

568 Erwin PM, López-Legentil S, González-Pech R, Turon X (2012a) A specific mix of
569 generalists: bacterial symbionts in mediterranean *Ircinia spp.* FEMS Microbiol
570 Ecol 79:619-637

571 Erwin PM, López-Legentil S, Turon X (2012b) Ultrastructure molecular phylogenetics
572 and chlorophyll a content of novel cyanobacterial symbionts in temperate
573 sponges. Microb Ecol 64:771-783

574 Erwin PM, Pita L, López-Legentil S, Turon X (2012c) Stability of sponge-bacteria
575 symbioses over large seasonal shifts in temperature and irradiance. App
576 Environ Microb 78:7358-7368

577 Fan L, Reynolds D, Liu M, Stark M, Kjelleberg S, Webster NS, Thomas T (2012)
578 Functional equivalence and evolutionary convergence in complex communities
579 of microbial sponge symbionts. P Natl Acad Sci USA 109:E1878-E1887

580 Garrabou J, Zabala M (2001) Growth dynamics in four mediterranean demosponges.
581 Estuar Coast Shelf S 52:293-303.

582 Hoppe WF (1988) Growth, regeneration and predation in three species of large coral
583 reef sponges. Mar Ecol Prog Ser 50:117-125

584 Koopmans M, Wijffels RH (2008) Seasonal growth rate of the sponge *Haliclona oculata*
585 (Demospongiae: Haplosclerida). Mar Biotechnol 10:502-510

586 Liu M, Fan L, Zhong L, Kjelleberg S, Thomas T (2012) Metaproteogenomic analysis of
587 a community of sponge symbionts. The ISME Journal 6:1515-1525

588 López-Legentil S, Ruchty M, Domenech A, Turon X (2005) Life cycles and growth rates
589 of two morphotypes of *Cystodytes* (Ascidiacea) in the Western Mediterranean.
590 Mar Ecol Prog Ser 296:219-228

591 Mariani S, Uriz MJ, Turon X (2005) The dynamics of sponge larvae assemblages from
592 northwestern Mediterranean nearshore bottoms. J Plankton Res 27:249-262

593 Marti R, Uriz MJ, Ballesteros E, Turon X (2004) Benthic assemblages in two
594 Mediterranean caves: species diversity and coverage as a function of abiotic
595 parameters and geographic distance. J Mar Biol Assoc UK 84:557-572

McClintock JB, Amsler CD, Baker BJ, Van Soest RWM (2005) Ecology of Antarctic marine sponges: an overview. Integr Comp Biol 45:359-368

McMurray SE, Blum JE, Pawlik JR (2008) Redwood of the reef: growth and age of the giant barrel sponge *Xestospongia muta* in the florida keys. Mar Biol 155:159–171

Palacín C, Giribet G, Carner S, Dantart L, Turon X (1998) Low densities of sea urchins influence the structure of algal assemblages in the Western Mediterranean. J Sea Res 39:281-290

Pansini M, Pronzato R (1990) Observations on the dynamics of a mediterranean sponge community. In: Rützler K (ed) New Perspectives in Sponge Biology: 3d International Sponge Conference, 1985. Smithsonian Institution Press, Washington DC, pp 404–415

Perez T, Garrabou J, Sartoretto S, Harmelin JG, Francour P, Vacelet J (2000) Mortalité massive d'invertébrés marins: un événement sans precedent en Méditerranée nord-occidentale. Life Sci 323:853-86.

Pérez-Porro AR, González J, Uriz MJ (2012) Reproductive traits explain contrasting ecological features in sponges: the sympatric poecilosclerids *Hemimycale columella* and *Crella elegans* as examples. Hydrobiologia 687:315-330

Pérez-Portela R, Palacín C, Duran S, Turon X (2007) Biological traits of three closely related species of *Pycnoclavella* (Ascidiacea) in the Western Mediterranean. Mar Biol 152:1031-1038

Reiswig HM (1974) Water transport, respiration and energetics of three tropical marine sponges. J Exp Mar Biol Ecol 14:231-249

Rohde S, Schupp J (2012) Growth and regeneration of the elephant ear sponge *Ianthella basta* (Porifera). Hydrobiologia 687:219-226

Rützler K (2012) The role of sponges in the Mesoamerican barrier-reef ecosystem, Belize. Adv Mar Biol 61: 211-271

Sebens KP (1987) The Ecology of indeterminate growth in animals. Ann Rev Ecol Evol Syst 18:371-407

Stabili L, Cardone F, Alifano P, Tredici SM, Piraino S, Corriero G, Gaino E (2012) Epidemic mortality of the sponge *Ircinia variabilis* (Schmidt, 1862) associated to proliferation of a *Vibrio* bacterium. Microb Ecol 64:802-813

Steveli JM, Sweat DE, Bert TM, Sim-Smith C, Kelly M (2011) Sponge mortality at Marathon and Long Key, Florida: patterns of species response and population recovery. Proceedings of the 63rd Gulf and Caribbean Fisheries Institute: 384-400

Tanaka K (2002) Growth dynamics and mortality of the intertidal encrusting sponge *Halichondria okadai* (Demospongiae, Halichondrida). Mar Biol 140:383-389

Taylor MW, Radax R, Steger D, Wagner M (2007) Sponge-associated microorganisms: evolution, ecology and biotechnological potential. Microbiol Mol Biol Rev 71: 295-347

Teixidó N, Pineda M. C, Garrabou J (2009) Decadal demographic trends of a long-lived temperate encrusting sponge. Marine Ecology Progress Series 375: 113-124.

Teixidó N, Garrabou J, Harmelin JG (2011) Low dynamics, high longevity and persistence of sessile structural species dwelling on mediterranean coralligenous outcrops. PLoS One 6(8): e23744.

Thacker RW (2005) Impacts of shading on sponge-cyanobacteria symbioses: a comparison between host-specific and generalist associations. Integr Comp Biol 45:369-376

Thacker RW, Freeman CJ (2012) Sponge–microbe symbioses: recent advances and new directions. Adv Mar Biol 62:58-111

Trussell GC, Lesser MP, Patterson MR, Genovese SJ (2006) Depth-specific differences in growth of the reef sponge *Callyspongia vaginalis*: role of bottom-up effects. Mar Ecol Prog Ser 323:149-158

Tsoukatou M, Hellio C, Vagias C, Harvala C, Roussis V (2002) Chemical defense and antifouling activity of three mediterranean sponges of the genus *Ircinia*. Z Naturforsch 57:161-171

Turon X (1990) Distribution and abundance of ascidians from a locality on the northeast coast of Spain. Mar Ecol PSZNI 11:291-308

655 Turon X, Becerro MA (1992) Growth and survival of several ascidian species from the
656 northwestern mediterranean. Mar Ecol Prog Ser 82:235-247

657 Turon X, Becerro MA, Uriz MJ, Llopis J (1996) Small-scale association measures in
658 epitbenthic communities as a clue of allelochemical interactions. Oecologia
659 108:351-360

660 Turon X, Tarjuelo I, Uriz MJ (1998) Growth dynamics and mortality of the encrusting
661 sponge *Crambe crambe* (Poecilosclerida) in contrasting habitats: correlation
662 with population structure and investment in defence. Funct Ecol 12:631-639

663 Uriz MJ, Rosell D, Martín D (1992) The sponge population of the Cabrera Archipelago
664 (Balearic Islands): characteristics, distribution, and abundance of the most
665 representative species. Mar Ecol PSZNI 13:101-117

666 Vacelet J, Gaino E, Gallissian MF, Vacelet E (1994) Bacterial attack of spongin
667 skeleton during the 1990 Mediterranean sponge disease. In: van Soest RWM,
668 van Kempen TMG, Braekman JC (eds) Sponges in
669 Time and Space; Biology, Chemistry, Paleontology. Balkema, Rotterdam,
670 pp 355–362

671 Van Soest RWM, Boury-Esnault N, Vacelet J, et al. (2012) Global Diversity of Sponges
672 (Porifera). PLoS One 7:e35105

673 Van Treeck P, Eisinger M, Müller J, Paster M, Schuhmacher H (2003) Mariculture trials
674 with mediterranean sponge species The exploitation of an old natural resource
675 with sustainable and novel methods. Aquaculture 218:439-455

676 Webster NS, Taylor MW (2011) Marine sponges and their microbial symbionts: love
677 and other relationships. Environ Microbiol 14:335-346

678 Wulff JL (1985) Patterns and processes of size change in Caribbean Demosponges of
679 branching morphology. In: Rützler K (ed) New perspectives in sponge biology.
680 Smithsonian Institution Press, Washington, pp 425–435

681 Wulff JL (1991) Asexual fragmentation, genotype success, and population dynamics of
682 erect branching sponges. J Exp Mar Biol Ecol 149:227-247

683 Wulff JL (1997) Mutualisms among species of coral reef sponges. Ecology 78: 146-159

684 Wulff JL (2005) Trade-offs in resistance to competitors and predators, and their effects
685 on the diversity of tropical marine sponges. J Anim Ecol 74: 313-321

686 Wulff JL (2006) Rapid diversity and abundance decline in a Caribbean coral reef
687 sponge community. Biol Conserv 127: 167-176

688 Wulff JL (2008) Life-history differences among coral rees sponges promote mutualism
689 or exploitation of mutualism by influencing partner fidelity feedback. Am Nat
690 171:597-609

691 Wulff JL (2012) Ecological interactions and the distribution, abundance and diversity of
692 sponges. Adv Mar Biol 61:273-344

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Figure legends

Figure 1. *In situ* photographs of monitored individuals of *Ircinia fasciculata* (top row), *I. variabilis* (middle row) and *I. oros* (bottom row) over time. Scale bars represent 3 cm.

Figure 2. Regression analyses of area-volume relationships for the three species. Lower right graph shows the three regression lines on the same plot using a log-log scale.

Figure 3. Average monthly growth rates of the three species over the study period. Temperature graphs are superimposed. Bars are standard errors.

Figure 4. Cross-correlation plots of monthly growth rate and temperature for the three species. Negative time lags measure the relationship of growth rate with temperature in the preceding months, and the reverse is true for positive lags. The curved lines mark the significance threshold ($P = 0.05$) of the correlation coefficient.

Figure 5. Regression analysis of mean overall growth rates and initial sponge volume for the three species.

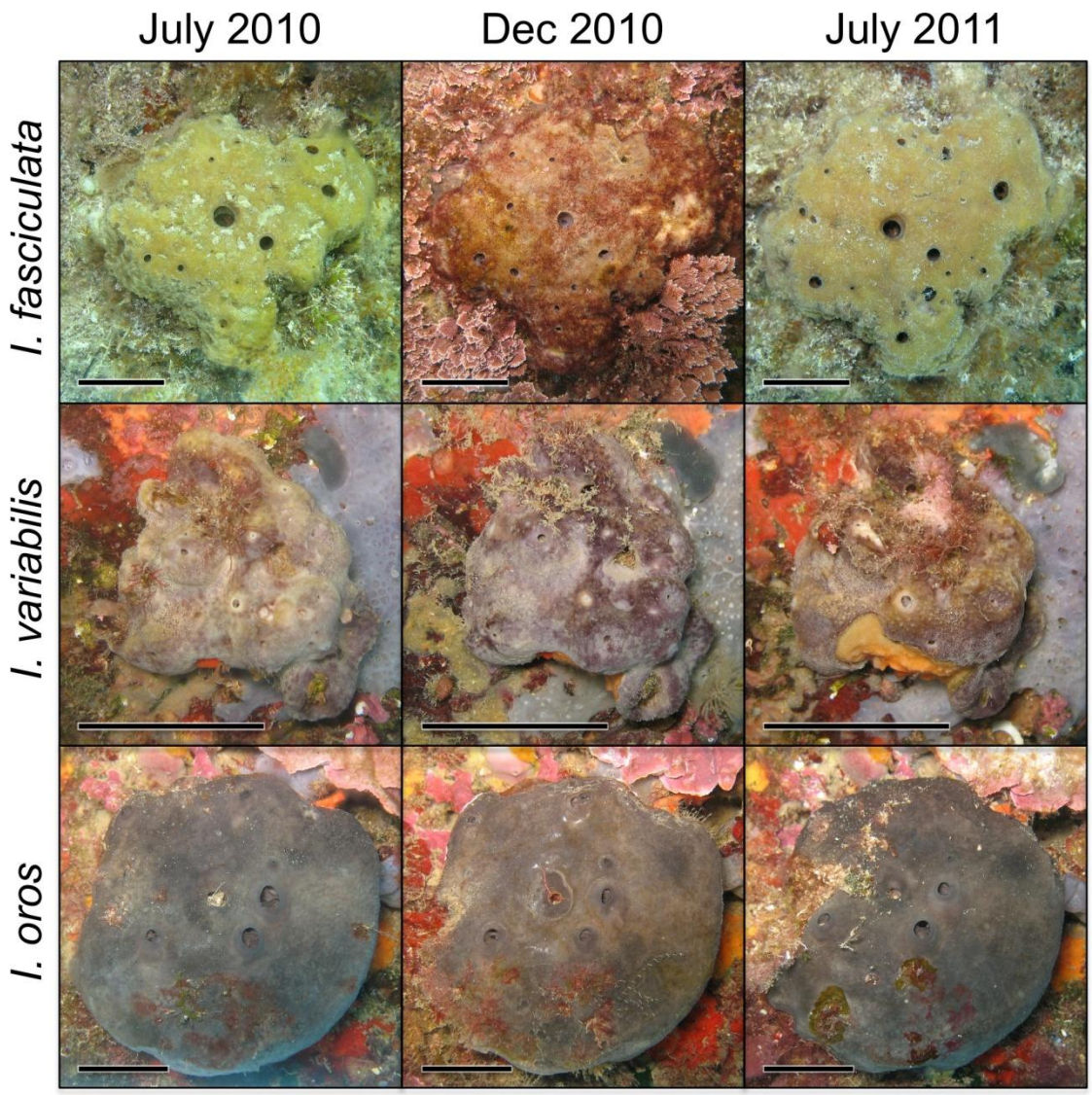
Figure 6. Plot of the mean number of oscula per unit volume (individual values averaged over months) in the three species. Bars are standard errors.

Figure 7. Plot of the mean circularity index (individual values averaged over months) in the three species. Bars are standard errors.

721

722 Figure 8. Time course of the fouling index of the three species over the study
723 period. Temperature graphs are superimposed. Bars are standard errors.

Figure



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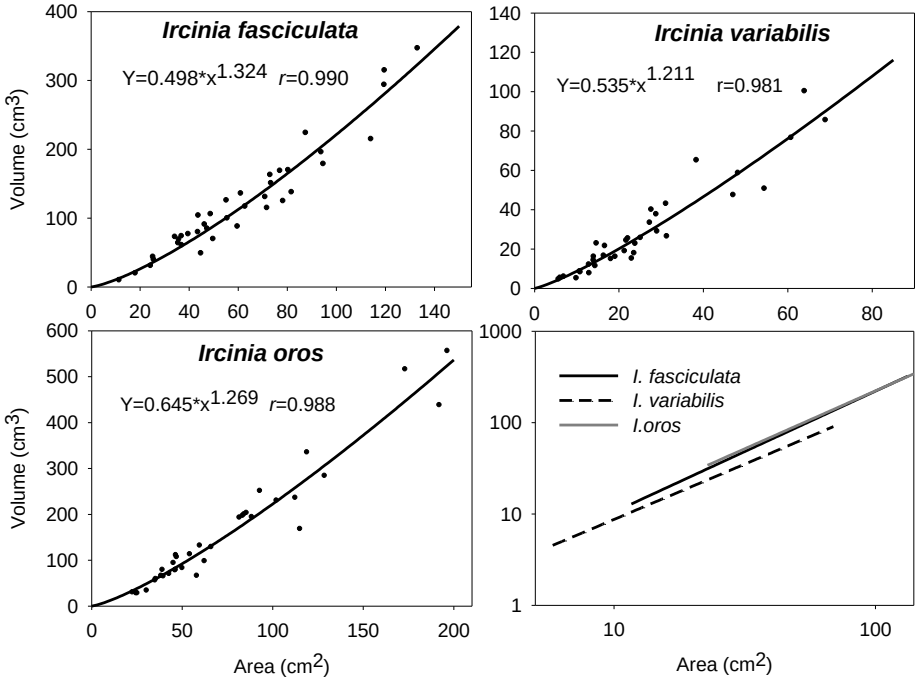

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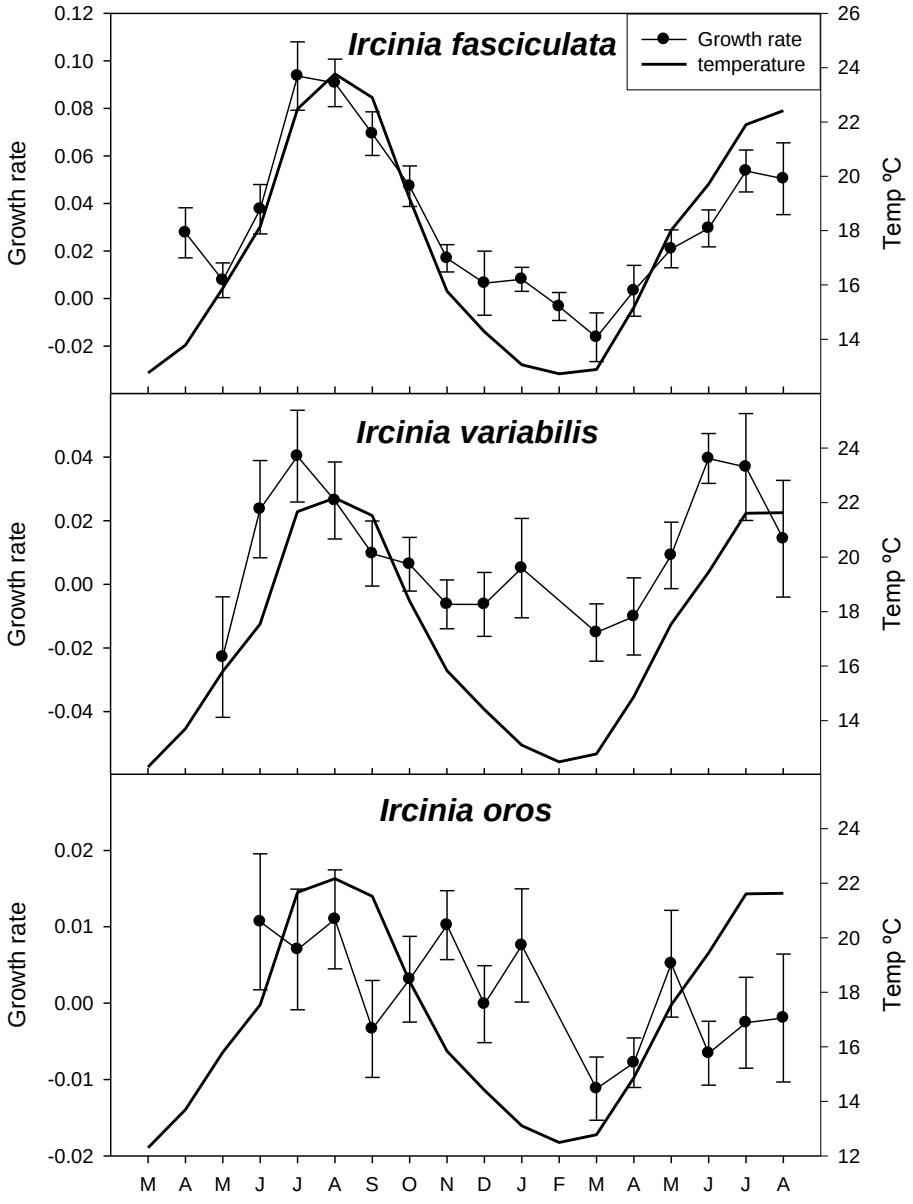

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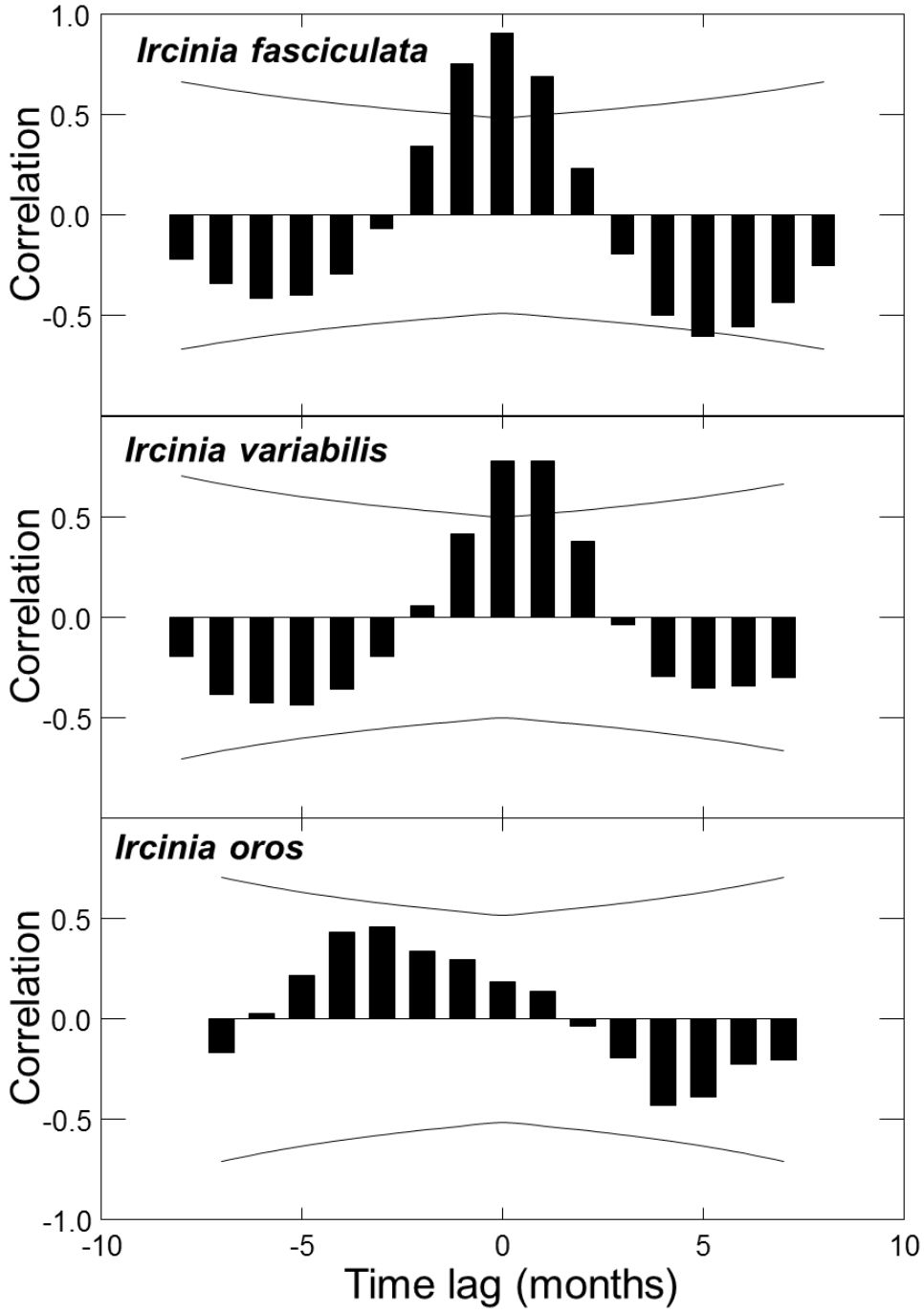

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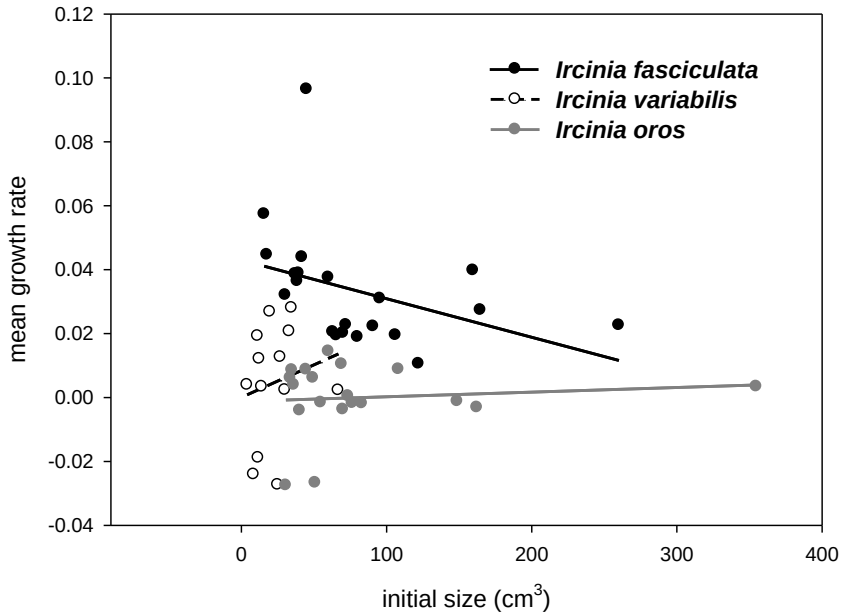

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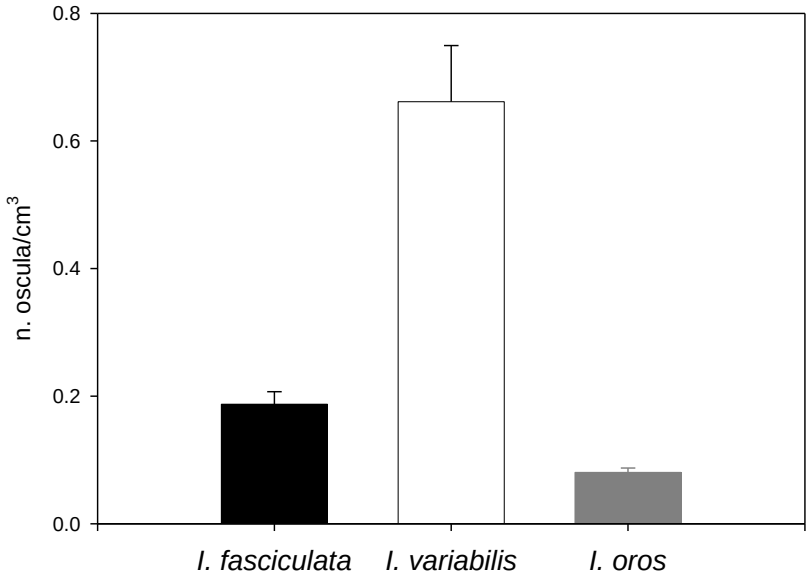

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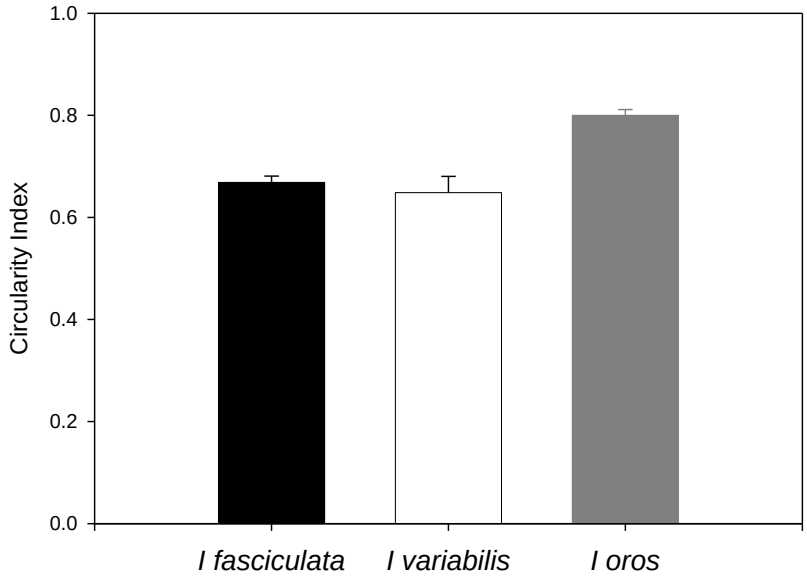

Figure 7. Plot of the mean circularity index (individual values averaged over months) in the three species. Bars are standard errors.

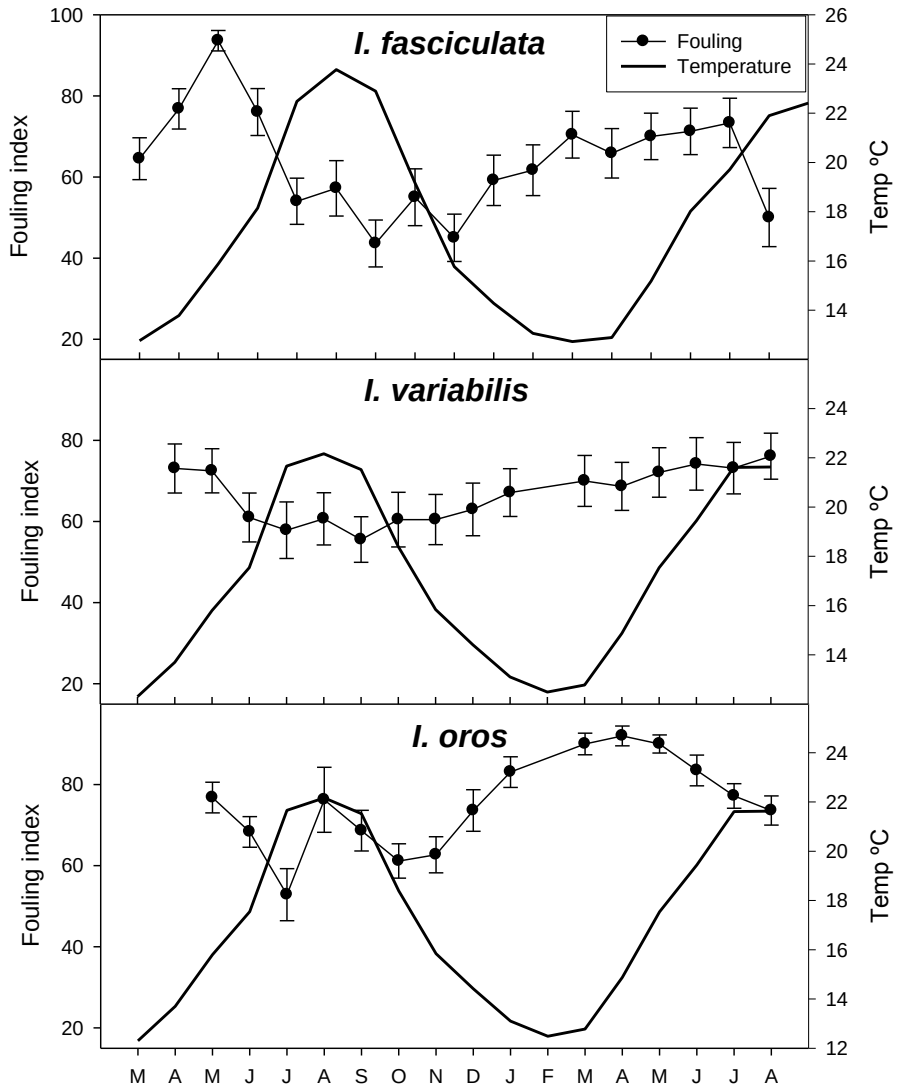

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Table

Table 1. Analysis of covariance for area/volume relationships in the three species with area as the covariate. (A) full model with interaction term. (B) restricted model without interaction term for *I. fasciculata* and *I. oros* only

(A)	SS	DF	MS	F	p
Species	8861.7	2	4430.9	8.2670	0.0005
Area	302278.7	1	302278.7	563.9868	<0.0001
Species*Area	18319.5	2	9159.7	17.0901	<0.0001
Error	55204.7	103	536.0		
(B)	**SS**	**DF**	**MS**	**F**	**p**
Species	206.0	1	206.0	0.2604	0.611
Area	772023.1	1	772023.1	975.8024	<0.0001
Error	55381.7	70	791.2		